BLOCK OF DELAYED RECTIFIER POTASSIUM CURRENT, I_k, BY COCAINE IN CAT VENTRICULAR MYOCYTES.

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It has been reported that cocaine prolongs the effective refractory period of ventricular muscle tissue. The mechanism for prolongation of the effective refractory period is not known. Because the delayed rectifier outward potassium current, I_k, is believed to play a major role in repolarizing the cardiac action potential, we hypothesized that cocaine blocks I_k. Block of I_k by cocaine was tested in cat ventricular myocytes using single suction pipette voltage-clamp techniques (normal HEPES buffer with Cd^{++} to block CA^{++} current, T=31-32°c). I_k tails (I_{tail}) were studied using clamp steps from -40 mV to selected test potentials (V_t, 0 to +70 mV, 750 ms), and results obtained with cocaine concentrations ranging from 1 to 60 μM were as follows:

Cocaine Dose (μM)	No. of cells studied	% Block in I_{tail} using test potential of + 40 mV
1	6	18.1 ± 7.6 *
3	5	28.7 ±12.8
10	4	71.1 ± 3.0
30	4	81.2 ± 6.1
60	2	88.7 ± 2.6

* Values are MEANS ± S.E.M.

Recovery from cocaine block was observed after washout of the drug. The time taken for recovery after exposure to 30μM cocaine ranged between 10 and 30 min. Block of I_{tail} occurred without any significant effect on the background potassium current, I_{ti}. Furthermore, block of I_{tail} occurred at cocaine concentrations lower than those required for blocking Na^+ channels in ventricular muscle. These results indicate that cocaine is a potent blocker of I_k in cat ventricular myocytes, and this effect may be responsible for cocaine-induced prolongation of the effective refractory period of ventricular muscle tissue.

HIGH AFFINITY OUABAIN BINDING OF THE ALPHA 2 ISOFORM OF Na,K-ATPase IN TRANSFECTED MAMMALIAN CELLS

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Ouabain and other cardiac glycosides bind to the alpha subunit of the Na,K-ATPase, and the pharmacologic effects of the cardiac glycosides may be related to isoform differences of this subunit. Three distinct alpha isoforms have been demonstrated, with the alpha 2 isoform (A2) found predominantly in cardiac and skeletal muscle, in adipose tissue, and in brain. Previous studies have suggested that rat A2 has a high affinity for ouabain in comparison to the rat alpha 1 isoform (A1), but independent assessment of this has been difficult because both isoforms often co-exist. Therefore, to evaluate the hypothesis that A2 has unique physiologic characteristics which may be important for cardiac cell function, we developed an expression system and constructed a new expression vector, PMT21-A2, containing rat A2 cDNA. Expression of PMT21-A2 was studied in mouse-derived NIH 3T3 cells, which express a ouabain-resistant A1, and which do not express endogenous A2. Northern analysis confirmed specific A2 mRNA production. Protein expression was documented by Western blotting using mouse monoclonal antibody against rat A2. Ouabain binding, measured using intact cell techniques, demonstrated high levels of specific binding in the A2 transfected cells. In contrast, non-A2 transfected cells showed virtually no specific binding. The level of ouabain binding for the A2 transfectants was similar to that reported for other ouabain-sensitive isoforms with a Kd of approximately 10^{-8} M. We conclude, first, that high levels of functional A2 protein expression can be achieved with this system. Second, because A2 and A1 can be independently analyzed in this system, our results confirm previous indirect evidence that rat A2 has high affinity ouabain binding properties.

BLOCK OF DELAYED RECTIFIER POTASSIUM CURRENT, I_K, BY TERFENADINE IN CAT VENTRICULAR MYOCYTES.

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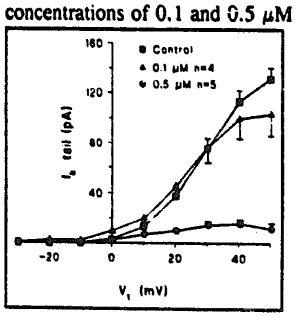
Terfenadine (Seldane™) is a widely prescribed non-sedating histamine H^1 receptor antagonist which has been rarely associated with torsades de pointes (TDP) and QT interval prolongation. The mechanism for these actions is not known and the electrophysiologic effects of terfenadine have not been reported. Since most of the drugs known to cause TDP block the delayed rectifier potassium current (I_k), we examined the effects of terfenadine on this current. Block of I_k by terfenadine was tested in cat ventricular myocytes using single suction pipette voltage-clamp techniques (normal HEPES buffer with Cd^{++} to block CA^{++} current, T=31-32°C). I_k tails were studied using clamp steps from -40 mV to selected test potentials (V_t, -30 to +50 mV, 750 msec). The results obtained with terfenadine concentrations of 0.1 and 0.5 μM were as follows (mean ±SE):



Terfenadine is a potent blocker of I_k. This action may be responsible for the rare cases of QT interval prolongation observed at high dosages and torsades de pointes seen with drug interactions that produce high concentrations of terfenadine.

CYCLOSPORINE A INCREASES THE INTRACELLULAR FREE CALCIUM CONCENTRATION IN ELECTRICALLY PACED ISOLATED RAT CARDIOMYOCYTES

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The effect of cyclosporine A on the mean intracellular free calcium concentration (Ca_i) in electrically paced single cardiomyocytes (MYO) from adult rats was studied. MYO were paced by electrical field stimulation with 2 Hz over a period of 30 min in the presence of 5 ug/ml cyclosporine A, the solvent methanol or Krebs Ringer Hepes buffer (KRH). Ca_i was measured by means of digital image processing of fura-2 fluorescence integrated over 1 sec. 30 min electrical field stimulation increased the Ca_i in KRH from 100.1 ± 7.1 (mean ± SEM) to 177.9 ± 8.8 nM Ca^{2+} (n=27 cells) and in methanol from 145.7 ± 8.0 to 200.6 ± 9.4 nM Ca^{2+} (n=28 cells). In contrast, there was a 3-fold increase in Ca_i with 5 ug/ml cyclosporine A from 128.8 ± 11.0 to 376.1 ± 14.4 nM Ca^{2+} (n=29 cells). The Ca_i during electrical stimulation was significantly higher with cyclosporine A than with methanol or with KRH ($p < 0.001$).

Conclusion:
The data provide direct evidence that cyclosporine A enhances the Ca_i in paced rat cardiomyocytes. These findings may be of importance in the consideration of a possible cardiotoxicity of cyclosporine A.